

**Human DNA
(cytosine-5)
Methyltransferase (Dnmt1)**



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www.neb.com



M0230S 032121213121

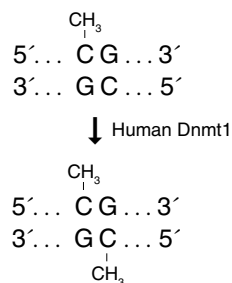
M0230S



50 units 2,000 U/ml Lot: 0321212

RECOMBINANT Store at -20°C Exp: 12/13

Methylation Site:



Description: Dnmt1 methylates cytosine residues in hemimethylated DNA at 5'...CG...3' (1,2). Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions (3-5). The bulk of the methylation takes place during DNA replication in the S-S-phase of the cell cycle (6).

Source: Dnmt1 is expressed from human Dnmt1 cDNA using a baculovirus expression system (1,7).

Supplied in: 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 200 mM NaCl, 1 mM dithiothreitol and 50% glycerol. Store at -20°C.

Reagents Supplied with Enzyme:
10X Dnmt1 Reaction Buffer, 100X BSA and 32 mM S-adenosylmethionine (SAM).

Reaction Conditions: 1X Dnmt1 Reaction Buffer, supplemented with 100 µg/ml BSA (supplied) and 160 µM S-adenosylmethionine (supplied). Incubate at 37°C.

1X Dnmt1 Reaction Buffer:

50 mM Tris-HCl
1 mM EDTA
1 mM dithiothreitol
5% glycerol
pH 7.8 @ 25°C

Unit Definition: One unit is the amount of enzyme required to catalyze the transfer of 1 pmol of methyl group to poly dI.dC substrate in a total reaction volume of 25 µl in 30 minutes at 37°C.

Quality Assurance: Purified free of contaminating endonucleases and exonucleases.

Storage of SAM: S-adenosylmethionine (SAM) (Sigma Catalog #A7007) is stored at -20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C (1) and should be replenished in reactions incubated longer than 4 hours. Methylation can be optimized by using fresh SAM.

Heat Inactivation: 65°C for 20 minutes.

Note: For DNA modification and protection applications, M.SssI (NEB #M0226) is preferred because it efficiently methylates both unmethylated and hemimethylated DNA substrates.

References:

1. Pradhan, S. et al. (1999) *J. Biol. Chem.* 274, 33002-33010.
2. Bacolla, A. et al. (1999) *J. Biol. Chem.* 274, 33011-33019.
3. Schmutte, C. et al (1998) *Biol. Chem.* 379, 377-388.
4. Laird, P.W. et al. (1995) *Cell* 81, 197-205.
5. Li, E. et al. (1992) *Cell* 12, 915-926.
6. Leonhardt, H. et al. (1992) *Cell* 71, 865-873.
7. Yen, R.W et al. (1992) *Nucleic Acids Res.* 20, 2287-2291.

CERTIFICATE OF ANALYSIS

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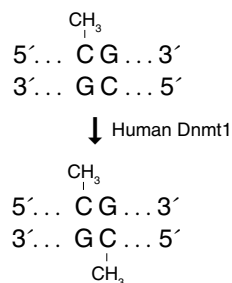
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